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Effect of Heavy metals on Morphological and Biochemical characteristics of *Albizia procera* (Roxb.) Benth. Seedlings

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ABSTRACT

An investigation was conducted to study the differential action of heavy metals such as Cd, As and Pb on morphological and biochemical parameters of *Albizia procera*. These heavy metals at all concentration (1, 5 and 10 ppm) exhibited significant reduction in chlorophyll, crude protein, amino acid and soluble sugar quantity in leaves. These biochemical parameters showed a positive correlation with leaf area, root length, shoot length and biomass of the plant,.Polyphenol, proline and ascorbic acid of leaves significantly increased over control but revealed negative correlation with root-shoot length, leaf area and biomass of plant at all treatment levels. Concentration dependent changes were observed in most of the parameters. Hence, the morphological and biochemical traits may serve to determine suitable bio-indicators of heavy metal pollution and also for the classification of the species as tolerant or sensitive to heavy metals.

Key words: *Albizia procera*- Biochemical indicator-Heavy metals- Toxicity

1. Introduction

Environment Pollution is one of the severe problems worlds facing today. Various efforts have been done for environmental restoration in India but still it seems to be a formidable task. Heavy metals are major environmental pollutant, which are discharged into the atmosphere from the burning of fossil fuels, release of industrial wastes and use of agrochemicals. Heavy metals are defined as elements with metallic properties (ductility, conductivity, stability as cations etc.) and an atomic weight > 20 or in other words, with a density higher than 5g cm^{-3}. (Weast,1984).

The concept that plants may be able to acclimatise with the incidences of pollution and contamination is now receiving greater attention, therefore present work has been carried out on the tolerance and adaptation of trees in metal polluted areas. It has been taken up to evaluate the effect of heavy metals such as cadmium, arsenic and lead on certain biochemical constituents and morphological features of *Albizia procera.*

2. Materials and Methods

A. procera seeds procured from Forest Research Institute, Dehradun, (Uttarakhand), India, were selected for uniformity of colour and size, soaked overnight in distilled water containing bavestine. Seeds were sown in polythene bags filled up with sand, soil and farm-yard manure (1:1:1) in Central Nursery of Forest Research Institute, and plants were irrigated with water once every two to three days. Treatment i.e., 1ppm, 5ppm and 10ppm were given after six

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months of the establishment of seedlings and subsequent treatments were given every week for four months. Three replicates were kept for each treatment along with control.

Cadmium supplied as $CdCl_2$, Arsenic as As_2O_3 and Lead as Pb $(CH_3COO)_2$. After completion of treatment for four months, leaf area, root-shoot length, biomass, biochemical parameters (chlorophyll. Crude protein, total free amimo acid, proline, ascorbic acid, soluble sugar and polyphenol of leaves were studied. All the biochemical parameters were estimated by using spectrophotometer (UV 2700).

Chlorophyll in leaves was estimated by Arnon (1945) method, soluble sugars in leaves were estimated by phenol-sulphuric acid method of Dubois *et al.* (1956). Polyphenol was measured by Schanderi method (1970). Total free amino acids were measured by the method of Moore *et al.,* (1948), soluble protein by Lowry *et al.*, (1971), proline by Bates *et al.*, (1973), ascorbic acid by Sadasivam *et al*, (1987). Leaf area was measured by using leaf area meter (GMT 7020) while root-shoot length and biomass were estimated following standard methods.

3. Results and Discussion

After six months of exposure, leaf samples of the plants species were analysed for morphological and biochemical parameters and impact of different heavy metals on these parameters was investigated.

3.1 Cadmium

The concentrations of metals adversely affected root-shoot length, leaf area and biomass of *A. procera.* Albizia seedling when supplied with heavy metals, Cd showed inhibition of growth and concentration dependent decrease in morphological parameters. 1ppm treatment of Cd caused reduction of 29.8% in root length, 1.5% in shoot length, and 15.02% in leaf area while 32.2% in biomass. Maximum reduction was caused by 10ppm of Cd treatment which showed reduction 50.8% in root length, 33.8% in shoot length, 37.7% in leaf area and 0.12% in biomass as compared to control. The interactions between metals, treatments and metal× treatments were highly significant ($P < 0.001$) (Table 1).

Leaves of *A. procera* exhibited reduction in total chlorophyll content at all treatments, over control. Inhibitory effects were gradually increasing with the increasing concentration ($P < 0.001$). 10ppm treatment of Cd caused maximum reduction in chlorophyll (27.6%), followed by 23.3% by 5ppm and 0.1% by 1ppm of treatment as compared to their control values. However, all interactions were highly significant ($P < 0.001$) (Table 2).

A concentration dependent increase in polyphenol content was observed ($P< 0.001$). 10ppm concentrations of Cd exhibited the maximum increase in polyphenol content (44%) as compared to control. Similarly concentration dependent increase was observed in proline content of leaves ($P< 0.001$). 10ppm of Cd exhibited enhancement in the proline content (0.125%) as compared to control. All interactions were found to be highly significant ($P<0.001$) (Table 2).

A concentration dependent decrease in crude protein over control was observed in the leaves. Maximum decrease found in 10ppm Cd (81%) followed by 5ppm (71.8%) and 1ppm (58.4%). However, all interactions were highly significant ($P < 0.001$) (Table 2). A gradual decrease in soluble sugar with increasing concentrations of metals was observed over control ($P < 0.001$). Maximum decrease was exhibited by 10ppm treatment (64.5%) followed by 5ppm

(51.05%) and 1ppm (39.3%). The interactions were found to be highly significant ($P < 0.001$) (Table 2).

A concentration dependent decrease in amino acid content over control was observed in the leaves. Maximum decrease found in 10ppm of Cd (68.3%). However, all interactions were highly significant ($P< 0.001$). A concentration dependent increase in ascorbic acid content was observed ($P< 0.001$). 10ppm treatments of Cd exhibited the maximum increase in ascorbic acid content (94.2%) as compared to control (Table 2).

3.2 Arsenic

Albizia seedling when supplied with heavy metals, As inhibited the growth and other morphological parameters. Maximum reduction was observed in case of 10ppm treatment of As 25% in root length, 36.4% in shoot length, 20.14% in leaf area and 75.5% in biomass as compared to control. The interactions between metals, treatment and metal × treatment were significant ($P < 0.001$) (Table 1). Leaves of *A. procera* exhibited reduction in total chlorophyll content at all concentration over control. Inhibitory effects were gradually increasing with the increasing concentration ($P < 0.001$). 10ppm concentration of As caused maximum reduction (72.5%) in chlorophyll, followed by (55.52%) in 5ppm and (54.7%) in 1ppm of treatments as compared to their control values. However, all interactions were highly significant ($P < 0.001$) (Table 2).

A concentration dependent increase in polyphenol content was observed ($P< 0.001$). 10ppm concentrations of As exhibited the maximum increase in polyphenol content (66.6%) as compared to control. Similarly concentration dependent increase was observed in proline content of leaves ($P< 0.001$). 10ppm of As exhibited 80.4% enhancement in the proline content as compared to control. All interactions were found to be highly significant ($P<0.001$) (Table 2). A concentration dependent decrease in crude protein over control was observed in the leaves. Maximum decrease found in 10ppm of As (82.6%) followed by 5ppm is (56.6%) and 1ppm (12.1%). However, all interactions were highly significant ($P < 0.001$) (Table 2). A gradual decrease in soluble sugar with increasing concentrations of metals was observed over control ($P < 0.001$). Maximum decrease was exhibited by 10ppm treatments (64.5%) followed by 5ppm (51.05%) and 1ppm (39.3%). The interactions were highly significant ($P < 0.001$) (Table 2). A concentration dependent decrease in amino acid content over control was observed in the leaves. Maximum decrease occurred at 10ppm of As treatments (39.5%). However, all interactions were highly significant ($P< 0.001$). A concentration dependent increase in ascorbic acid content was observed ($P< 0.001$). 10ppm treatments of As exhibited the maximum increase in ascorbic acid content (99.9%) as compared to control.

3.3 Lead

A. procera seedling when supplied with Pb, revealed concentration dependent reduction in morphological parameters. Treatment of 10ppm of Pb showed maximum reduction i.e.50% in root length, 42.2% in shoot length, 44.7% in leaf area and 32.4% in biomass as compared to control. ($P < 0.001$) (Table1).

Leaves of *A. procera* exhibited reduction in total chlorophyll content at all treatments of Pb over control. Inhibitory effects were gradually increasing with the increasing concentration ($P < 0.001$). 10ppm treatment of Pb caused 69.3% reduction in chlorophyll, 89.8% in 5ppm and 28.09% in 1ppm treatments of Pb as compared to their control values. ($P < 0.001$) (Table2).

A concentration dependent increase in polyphenol content was observed (P< 0.001). 10ppm treatments of Pb exhibited the maximum increase in polyphenol content (28.6%) as compared to control. Similarly metal concentration dependent increase was observed in proline content of leaves (*P*< 0.001). 10ppm of Pb exhibited 112.8% enhancement in the proline content while 14.1% in 1ppm treatment as compared to control. All interaction were found to be highly significant (*P*<0.001) (Table 2).

A concentration dependent decrease in crude protein over control was observed in the leaves. Maximum decrease found in 10ppm of Pb (80.7%) followed by 5ppm (29.8%) and (11.56%) of 1ppm treatment. All interactions were highly significant (*P* < 0.001) (Table 2). A gradual decrease in soluble sugar with increasing concentrations of metals was observed over control (*P* < 0.001). Maximum decrease was exhibited by 10ppm treatments i.e. 40.08% followed by 5ppm (33.6%) and 1ppm (12.4%). (Table2).

Table 1: Data showing the impact of different treatment of heavy metals on root length shoot length, leaf area and biomass of *Albizia Procera*

Treatments(mg/L)	Metals			CD at. 5%
	Cd	As	Pb	
Root length(cm)				
Control	59 ± 2.00	52 ± 4.00	56 ± 4.00	A_1 = 3.133* A_2 = 3.617*** $A_1 \times A_2$ = 6.266*
1	42 ± 2.00	48 ± 4.00	43 ± 9.00	
5	37 ± 1.00	45 ± 5.00	38 ± 2.65	
10	29 ± 1.00	39 ± 3.00	28 ± 2.00	
Shoot length (cm)				
Control	130 ± 5.00	129 ± 1.59	90 ± 8.66	A_1 = 3.371*** A_2 = 3.892*** $A_1 \times A_2$= 6.742***
1	128 ± 2.00	126 ± 4.00	80 ± 4.00	
5	92 ± 2.00	65 ± 3.00	56 ± 2.00	
10	86 ± 4.00	82 ± 4.00	52 ± 1.00	
Leaf area ($cm^{2)}$				
Control	25.56 ± 0.11	26.56 ± 0.02	25.56 ± 0.58	A_1 = 0.1789*** A_2 = 0.2066*** $A_1 \times A_2$ = 0.3578***
1	21.72 ± 0.48	26.22 ± 0.01	24.59 ± 0.07	
5	20.92 ± 0.01	23.41 ± 0.04	18.51 ± 0.10	
10	15.91 ± 0.03	21.21 ± 0.02	14.11 ± 0.06	
Biomass(gm/plant)				
Control	278.16 ± 0.10	247.65 ±1.31	205.08 ± 0.02	A_1 = 0.5277*** A_2 = 0.6093*** $A_1 \times A_2$= 1.055***
1	188.57 ± 0.07	132.57 ± 0.02	198.29 ± 0.16	
5	185.47 ± 0.06	112.2 ± 0.20	188.05 ± 0.04	
10	180.23 ± 1.85	60.53 ± 0.01	138.58 ± 0.09	

Values expressed are means ±standard deviation of three separate experiments; A_1 = CD at 5% by different metal comparisons; A_2= CD at 5% by different treatments comparisons; A_1 X A_2 = CD at 5% by comparing metal X different treatments; *Significance at .05%; ** Significance at .01%; ***Significance at .001%.

A concentration dependent decrease in total total free amino acid content over control was observed in the leaves. Maximum decrease occurred at 10ppm of Pb treatment (39.05%). However, all interactions were highly significant (*P*< 0.001). A concentration dependent increase in ascorbic acid content was observed (*P*< 0.001) but the interactions between metal

and treatment were non-significant. 10ppm treatment of Pb exhibited the maximum increase in ascorbic acid content (131.1%) as compared to control. It was also observed by the present study that morphological and biochemical parameters of *A. Procera* seedling was highly affected by Lead treatment at different concentrations, followed by Cadmium and Arsenic (Pb> Cd>As).

Table 2: Data showing the impact of different treatments of heavy metals on biochemical constituents of *Albizia procera*

Treatments(mg/L)	Metals			CD at. 5%
	Cd	As	Pb	
Chlorophyll (mg/g) fresh wt.				
Control	1.995 ± 0.175	2.053 ± 0.095	1.673 ± 0.285	A_1 = 0.1073*** A_2 = 0.1239*** $A_1 \times A_2$= 0.2147***
1	1.993 ± 0.175	0.930 ± 0.050	1.203 ± 0.015	
5	1.530 ± 0.075	0.913 ±0.035	1.060 ± 0.020	
10	1.443 ± 0.015	0.563 ± 0.205	0.513 ± 0.095	
Polyphenol (%)				
Control	2.915 ± 0.505	2.530 ± 0.080	3.590 ± 0.040	A_1 = 0.1186*** A_2 = 0.137*** $A_1 \times A_2$ = 0.2373***
1	2.515 ± 0.015	2.995 ± 0.015	4.195 ± 0.065	
5	3.005 ± 0.025	3.700 ± 0.030	4.465 ± 0.011	
10	4.200 ± 0.020	4.215 ± 0.005	4.620 ± 0.010	
Proline (mg/g)				
Control	0.098 ± 0.0015	0.097 ± 0.001	0.078 ± 0.001	A_1 = 2.352*** A_2 = 2.715*** $A_1 \times A_2$ = 4.704***
1	0.101 ± 0.002	0.104 ± 0.005	0.089 ± 0.010	
5	0.171 ± 0.0015	0.163 ± 0.001	0.106 ± 0.002	
10	0.221 ± 0.030	0.175 ± 0.004	0.166 ± 0.002	
Crude Protein (mg/g)				
Control	47.75 ± 0.020	48.51 ± 0.765	47.75 ± 0.025	A_1 = 1.081*** A_2 = 1.249*** $A_1 \times A_2$ = 2.163***
1	19.83 ± 2.010	42.63 ± 0.535	42.22 ± 0.025	
5	13.38 ± 1.500	21.05 ± 0.530	33.50 ± 1.054	
10	9.020 ± 1.100	8.405 ± 2.295	9.170 ± 2.650	
Ascorbic acid (mg/g)				
Control	3.280 ± 0.0057	2.913 ± 0.365	2.915 ± 0.365	A_1 = 0.364** A_2 = 0.4203*** $A_1 \times A_2$ = 0.7281^{ns}
1	4.190 ± 0.910	3.640 ± 0.006	4.183 ± 0.185	
5	6.005 ± 0.185	4.190 ± 0.910	4.913 ± 0.182	
10	6.370 ± 0.180	5.825 ± 0.365	6.737 ± 0.545	
Soluble sugar (mg/g)				
Control	36.580 ± 0.010	36.555 ± 0.035	36.585 ± 0.005	A_1 = 1.036*** A_2 = 1.1972*** $A_1 \times A_2$ = 2.073***
1	22.170 ± 0.006	21.045 ± 0.845	32.015 ± 3.245	
5	17.905 ± 1.045	16.725 ± 0.985	24.270 ± 2.340	
10	12.965 ± 1.055	11.760 ± 0.500	21.920 ± 0.001	
Total free amino acid (mg/g)				
Control	11.24 ± 0.410	10.92 ± 0.015	10.83 ± 0.020	A_1 = 0.1159*** A_2 = 0.1338*** $A_1 \times A_2$ = 0.2319***
1	4.465 ± 0.015	8.240 ±0.020	8.240 ± 0.021	
5	4.285 ± 0.005	7.260 ± 0.030	7.260 ± 0.020	
10	3.560 ± 0.050	6.600 ± 0.200	6.600 ± 0.200	

Values expressed are means ±standard deviation of three separate experiments; A_1 = CD at 5% by different metal comparisons; A_2= CD at 5% by different treatments comparisons; A_1 X A_2 = CD at 5% by comparing metal X different treatments; *Significance at .05%; ** Significance at.01%; ***Significance at .001%, ^{NS}Non-Significant

Result indicates that heavy metals decreased chlorophyll content in *A. procera* leaves with increasing concentrations of metals. Reduction in chlorophyll content may be due to the interference of all the metals with chlorophyll synthesis and fat metabolism, inhibiting root-shoot growth, photosynthesis, nutrient uptake, leaf area, biomass etc. (Pollacco, 1977).

Polyphenol occurs in all plant parts and offer resistance against diseases and pests. In present study polyphenol content is gradually increasing with the increasing concentration of As and Pb, but in Cd decreased the content at 5ppm treatment and again increased in 10ppm treatment of Cd. It has been found that polyphenols concentration can alter with the altered natural environmental conditions, and increases the tissues surrounding a wound (Stafford, 1987). In the present study, high level of polyphenols may be due to severe foliar injuries like chlorosis and necrosis caused by metal toxicity on plants. Increased level of polyphenols may be considered as signal of stress due to pollution or some other injury. 10ppm treatment of Cd, As and Pb caused maximum increase up to 44.08%, 66.6% and 28.69% respectively as compared to control.

Proline, a total free amino acid accumulated in plants when they experience moisture stress conditions and decline on release of stress (Pokhriyal *et al.*, 1985). Proline accumulation provides high stability in drought induced stress. Results indicate that Cd treated plant accumulated maximum proline content at 10ppm, followed by 10ppm treatments of As and Pb. Besides, control plants maintain a similar level of proline content.

Heavy metals reduced crude proteins in agricultural crops and in this study the effect was much pronounced on forestry tree species as compared to agricultural crops (Hemalatha *et al.*, 1997). However, maximum reduction occurred at 10ppm of As (82.67%). The decrease caused either by reduced *denovo synthesis* or by an increased decomposition of crude protein to amino acid, it may also serve as suitable indicator of pollution level (Todd *et al.*, 1961). The decrease in protein could be understood that metal in all likelihood would interfere with sulphur containing amino acid and crude protein resulted in decreased protein content (Somasundaram *et al.*, 1994). Amino acids play a central role in plant primary metabolism. Being early products of photosynthesis and nitrogen assimilation, they represent an important link between nitrogen and carbon metabolism (Durzan & Steward, 1983).

Ascorbic acid, a natural antioxidant plays an important role in pollution tolerance, and a direct relationship between endogenous levels of ascorbic acid and plant susceptibility to pollutant has been established (Keller *et al.*, 1977). Ascorbic acid maintains the stability of cell membranes during pollution stress and scavenges cytotoxic free radicals. Other reports show that plants in the polluted area either produce or consist of initially a higher amount of ascorbic acid. Increase of ascorbic acid content in leaves with increasing concentration of metals show susceptibility to heavy metals.

Soluble Sugar, an important constituent is manufactured during photosynthesis and breakdown during respiration by plants. All metals have decreased the content with increasing concentration as reported in agricultural crops also (Hemalatha *et al.*, 1997). Such inhibition of photosynthesis in higher plants by heavy metals has been reported (Bazzaz *et al.*, 1975). The low sugar levels may be due to lowered synthesis or diversion of the metabolites to other synthesis processes.

Correlation coefficient calculated (Table. 3) for chemical parameters and seedling growth showed leaf area, root length, shoot length and biomass of seedlings were significantly and positively correlated with chlorophyll, crude protein, soluble sugar and total free amino acid

while root length, shoot length, biomass were negatively correlated with polyphenol, proline and ascorbic acid. Similar results were observed with leaf area also, except polyphenol which could not be significantly correlated.

Table 3: Correlation coefficient 'r' between seedling growth and biochemical parameters.

	leaf area	root length	Shoot length	biomass	chlorophyll	protein	Amino acid	proline	Total sugar	Ascorbic acid	polyphenol
leaf area	1										
Root length	0.862***	1									
shoot length	0.644**	0.590*	1								
biomass	0.331	0.474*	0.483*	1							
chlorophyll	0.449	0.517*	0.710***	0.827***	1						
protein	0.792***	0.801***	0.475*	0.636**	0.4727*	1					
Amin	0.698**	0.790	0.302	0.458	0.2600	0.860	1				

oacid	*	***		*		***					
proline	-0.494*	-0.458*	-0.101	-0.0144	0.0372	-0.410	-0.497*	1			
total sugar	0.587**	0.690***	0.377	0.788***	0.576**	0.869***	0.8302***	-0.423	1		
ascorbic acid	-0.872***	-0.848***	-0.581*	-0.491*	-0.545*	-0.859***	-0.757***	0.4333	-0.724***	1	
polyphenol	-0.531*	-0.473*	-0.769***	-0.407	-0.607*	-0.288	-0.208	0.2583	-0.273	0.42	1

*Significance at 0.05; ** Significance at 0.01; ***Significance at 0.001

4. Conclusions

It may be concluded that there is a direct relationship between the concentration of heavy metals and morphological and biochemical responses of plants and chemical characteristics of soil. Metabolically, physiological, biochemical responses of plants to heavy metal concentration can be viewed as potentially adaptive changes that decline the operation of metabolic regulatory mechanisms which favours the functioning of the plants during or after stress. Thus these parameters can serve as indicators of heavy metal pollution. Finally, data which is generated through this study will be very helpful in detecting the lethal levels of heavy metals for particular plant species, its tolerance and remediation capacity.

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